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Integrated report in lymphoproliferative disorders

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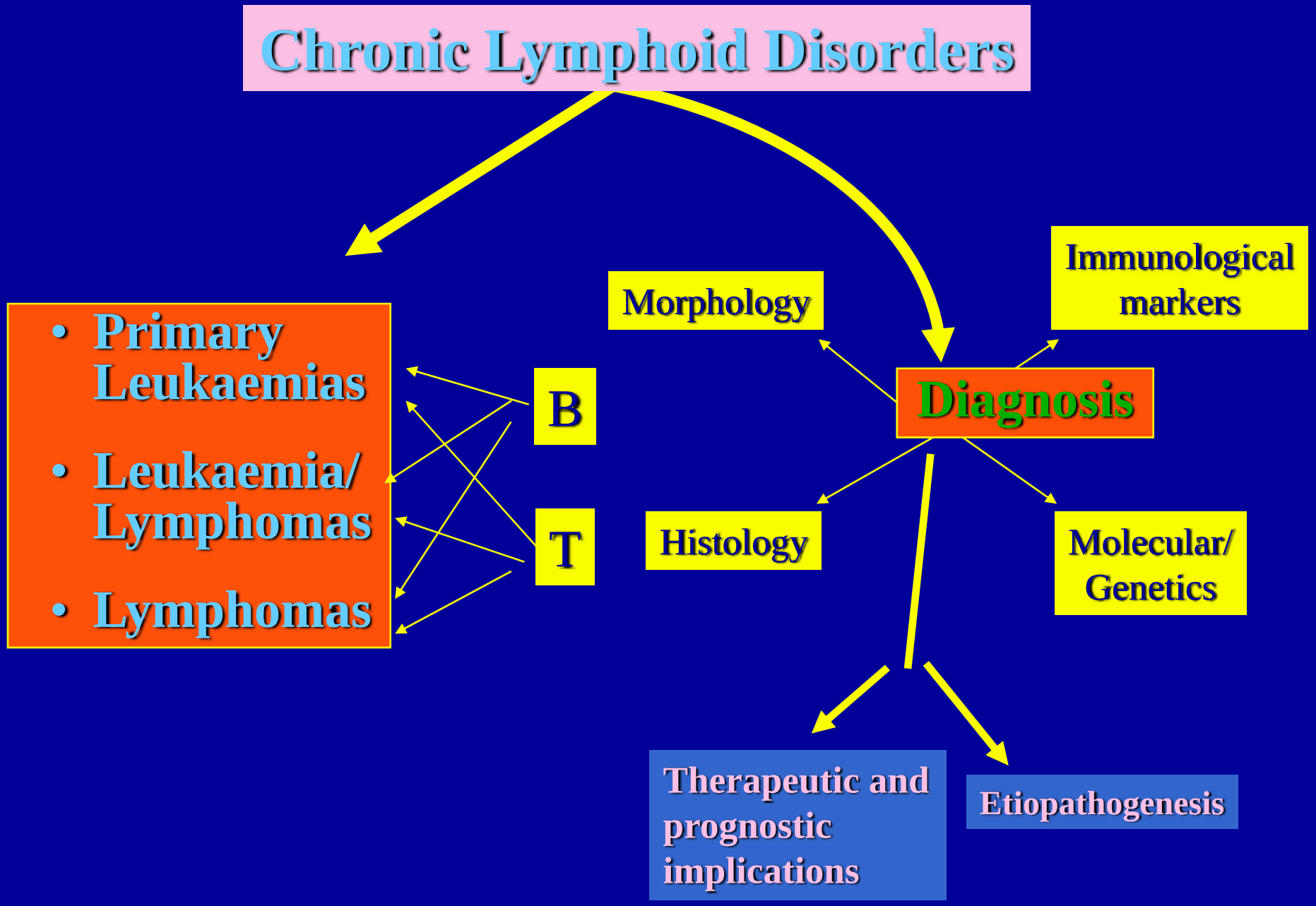

Chronic Lymphoid Disorders
• Primary Leukaemias
• Leukaemia/ Lymphomas
• Lymphomas
B
T
Morphology
Immunological markers
Diagnosis
Histology
Molecular/ Genetics
Therapeutic and prognostic implications
Etiopathogenesis

Historical background

1970´s and 1980´s

The diagnosis of CLPD was based on morphology, histology and limited immunophenotyping (IHC/FC)

1990´s FAB and REAL Classifications

Advances in immunophenotyping such as the recognition of new molecules characteristic of certain CLPD and improvement on technology (FC and IHC) allowed a better characterisation of the CLPD

Historical background

2000´s

2001 and 2008 WHO classifications

Incorporate in the diagnosis genetic abnormalities (gene deletions, mutations and/or translocations) specific for certain CLPD (i.e. mantle-cell lymphoma) or with a prognostic impact such as *TP53* dysfunction

Consider other molecular changes such as mutational analysis of the *IGVH* genes, stereotyped sequences, etc that provide insights into the cell origin where the neoplastic transformation has taken place as well as prognosis in some diseases

Integrated report

There is a need for an integrated report ?. YES

What the integrated report should provide to the clinician?

The diagnosis of a disease entity according to the WHO

Within a disease itself, the subcategory

When applicable information on prognosis

What the clinician should provide to the laboratory?

Full and comprehensive clinical data and adequate specimens

Integrated report in CLPD

At which stages of the disease an integrated report is required?

At diagnosis (presentation)

At relapse and/or progression (there may be transformation, changes on prognostic markers, ie acquisition of *TP53 deletion*)

? MRD

Tools for disease classification and stratification of CLPD

Morphology and immunophenotype are the first steps in the leukaemic CLPD as they allow to distinguish B from T cell disorders and establish the diagnosis in a substantial proportion of cases

Histology and IHC of the tissues affected is important in cases presenting with nodal disease without leukaemia or frank bm involvement

Cytogenetics/FISH play a key role in the diagnosis of some lymphomas (MCL or FL) and provide relevant prognostic information (CLL)

Southern-blot or PCR analysis of the TCR chain genes in T-cell lymphocytosis and mutational analysis of the *IgHV* in CLL

Miscellaneous

Chronic lymphocytic leukaemia (CLL) and CD5+ B-cell disorders

Morphology and immunophenotype may be typical or atypical of CLL

Prognostic tests if typical CLL is confirmed by the above:

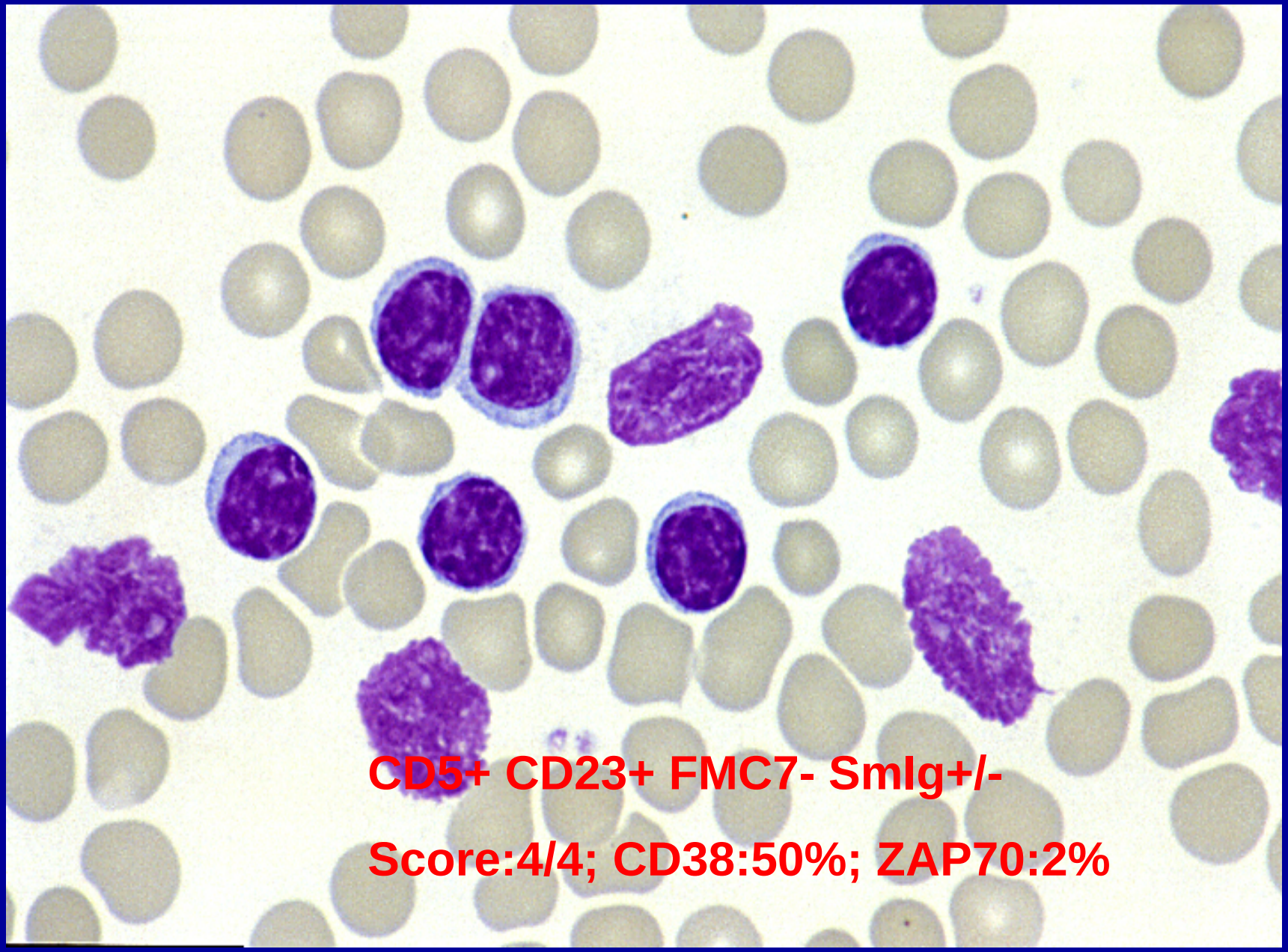
CD38 and ZAP70 expression (expression is unfavourable)

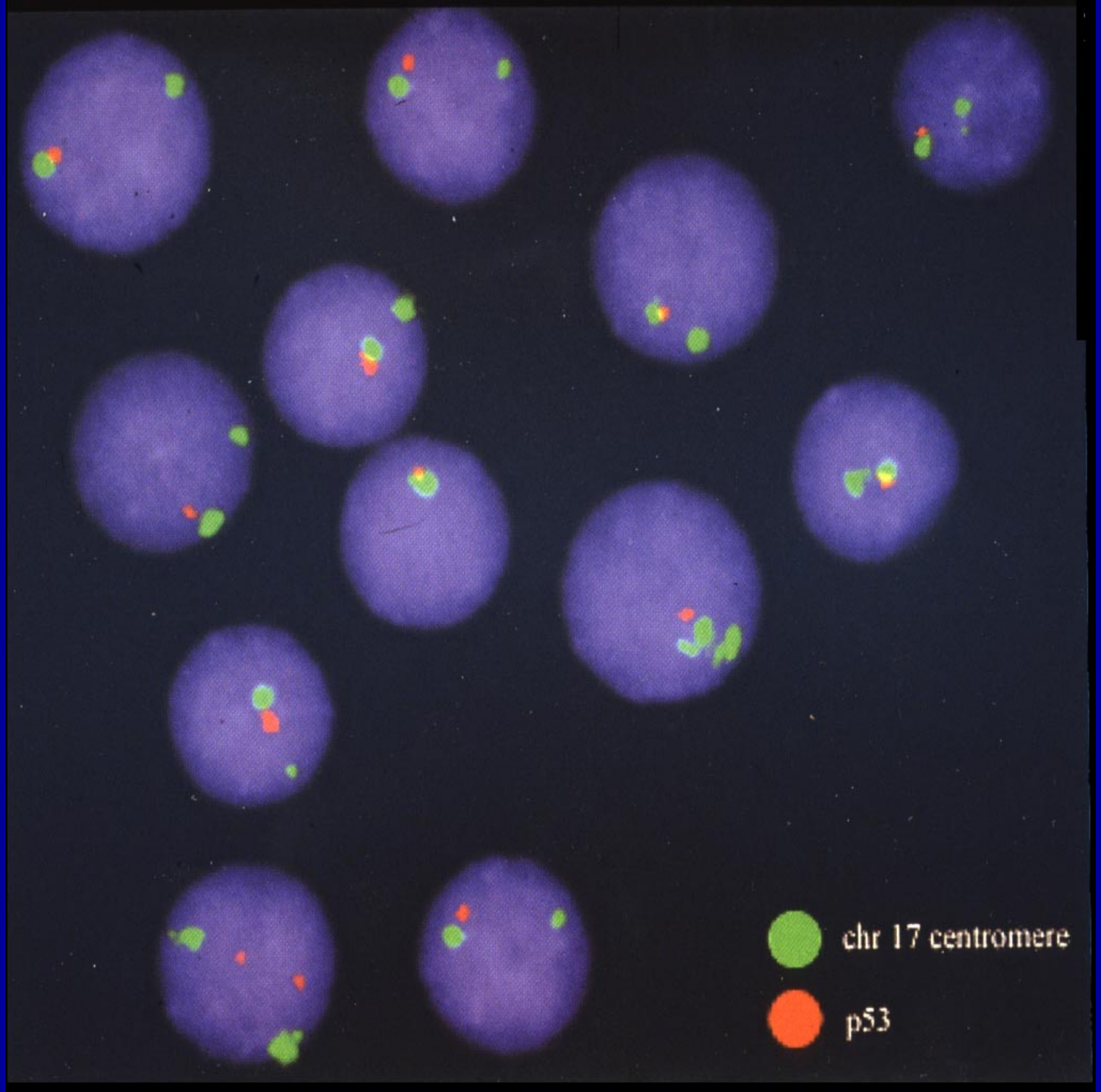
FISH for del13q14 , trisomy 12, del11q (*ATM)* and del17p *(TP53)*

(del 11q and del17p unfavourable)

Mutational analysis of the *IgVH* and family VH usage

unmutated (>98% homology) and VH3-21 (unfavourable)


CD5+ CD23+ FMC7- SmIg+/-
Score:4/4; CD38:50%; ZAP70:2%


chr 17 centromere
p53

Integrated report

Morphology : small lymphocytes with clumped chromatin; smudge cells

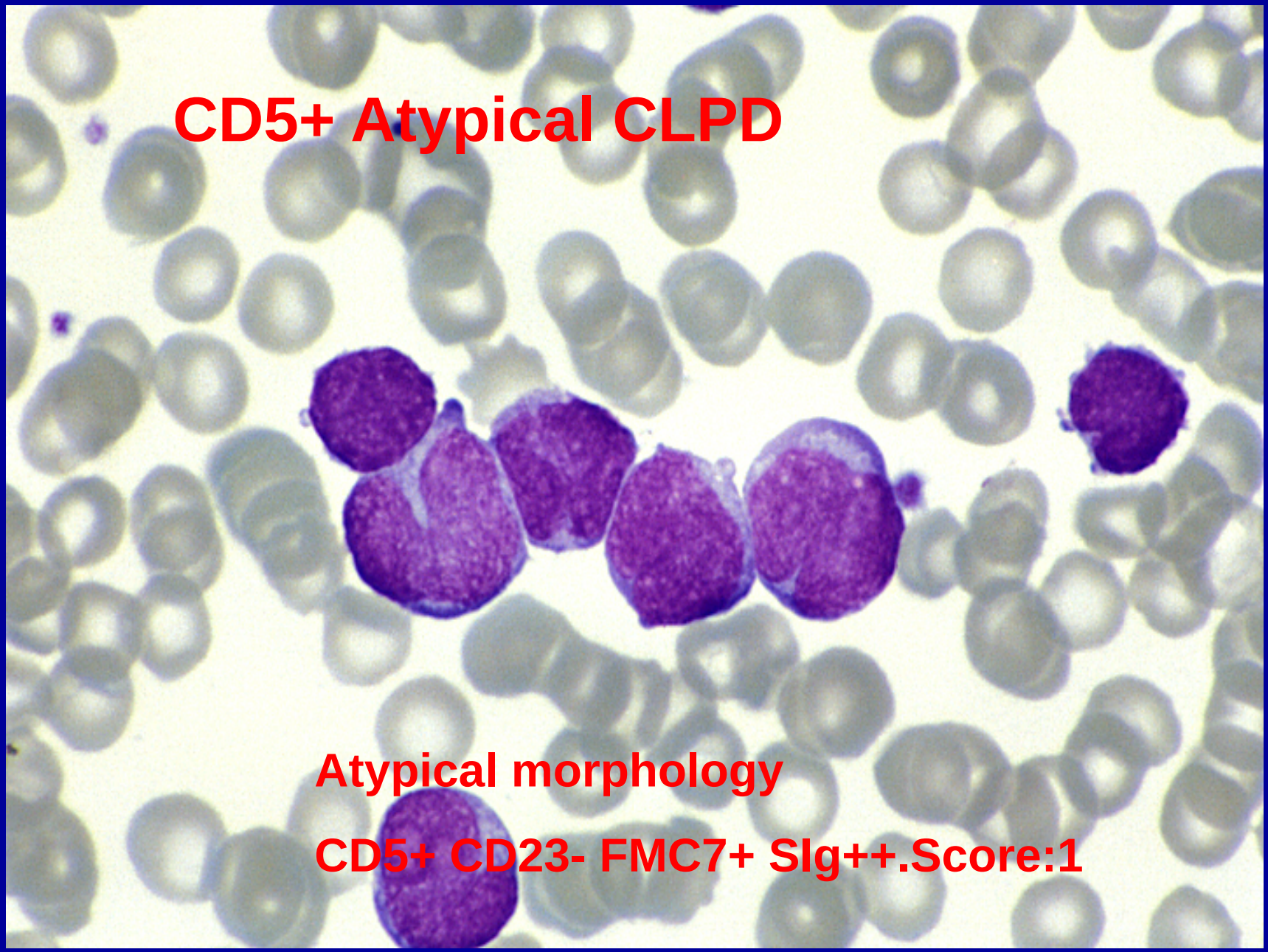
Immunophenotype: 90% of cells in the lymphocyte gate had a CLL phenotype CD5+ CD23+ FMC7- weak SmIg. CD38 was positive and ZAP-70 negative

FISH: no evidence of del13q14 , trisomy 12 or del11q (*ATM*)

90% of cells had del17p *(TP53)*

Molecular analysis: *IgVH* unmutated (99% homology to the germ line)

Diagnosis: Chronic lymphocytic leukaemia. CD38 expression, *TP53* deletion and unmutated *IgVH* are unfavourable prognostic markers factors


CD5+ Atypical CLPD
Atypical morphology
CD5+ CD23- FMC7+ Slg++.Score:1

Morphology and immunophenotype are atypical of CLL

CD5+ B-cell lymphoproliferative disorder non-CLL

Is it relevant to investigate prognostic tests for CLL?:

CD38 and ZAP70 expression (positive are unfavourable)

FISH for del13q14 , trisomy 12, del11q (*ATM)* and del17p *(TP53)*

Mutational analysis of the *IgVH* and family VH usage

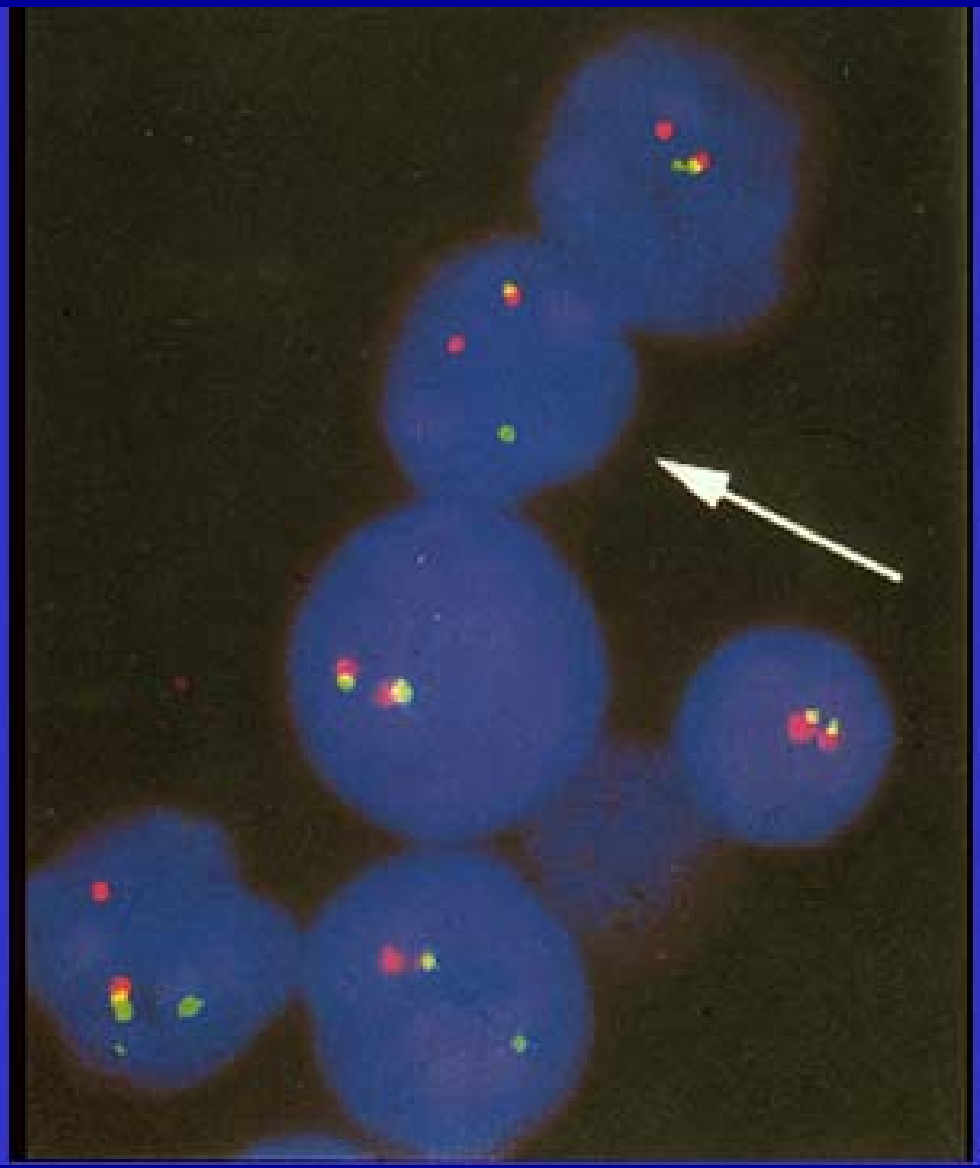
The answer is likely NO

Further tests to be carried out are

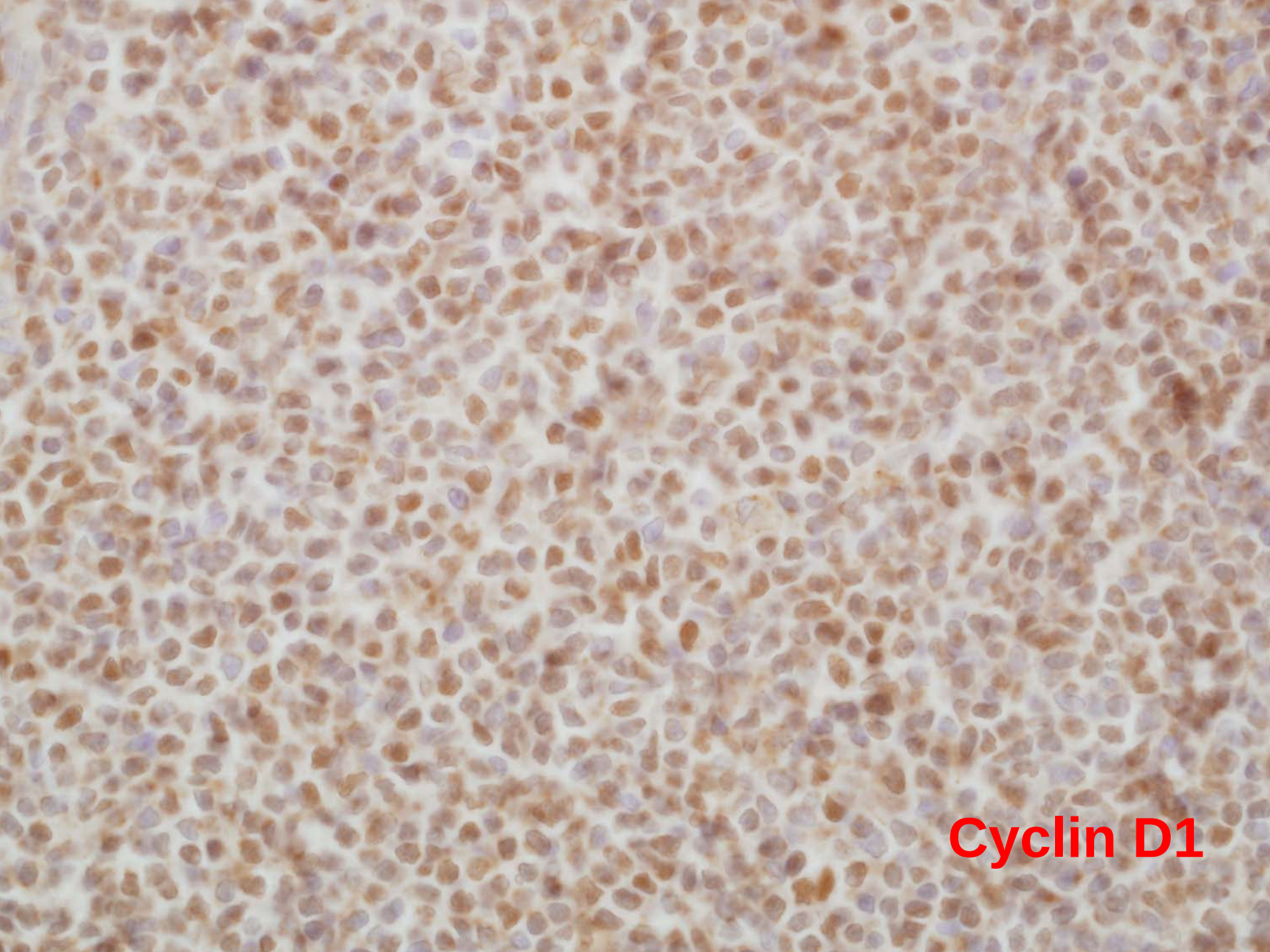
FISH for the t(11;14)(q23;q32)

Cyclin D1 stain in bone marrow if tissue histology is not possible

? Proliferative rate (Ki-67 or MIB)



FISH for *BCL-1* rearrangement


Cyclin D1

Integrated report

Morphology: medium size lymphocytes with speckled chromatin and nuclear indentations

Immunophenotype: 80% of cells in the lymphocyte gate have a CD5+ CD23- FMC7+ strong SmIg (CLL score 1)

FISH: 50% of cells have the t(11;14) with bcl-1 rearrangement

Bone marrow histology: diffuse infiltration by lymphoid cells CD5+ CD23- and with cyclin D1 expression. MIB-1= 5%

Diagnosis: Mantle-cell lymphoma in leukaemic phase non-blastoid with low proliferative rate

Integrated report in CLPD with circulating villous lymphocytes

(Hairy cell leukaemia (HCL), HCL-variant, SMZL/SLVL)

HCL can easily be distinguished from HCL-variant and SMZL by its distinct and unique morphology and immunophenotypic profile

Problems arise to distinguish HCL-variant from typical SMZL and diffuse red pulp SMZL

The need for an integrated report compounding morphology, immunophenotype, bone marrow histology and to some extend cytogenetics is well illustrated in these conditions

Integrated report

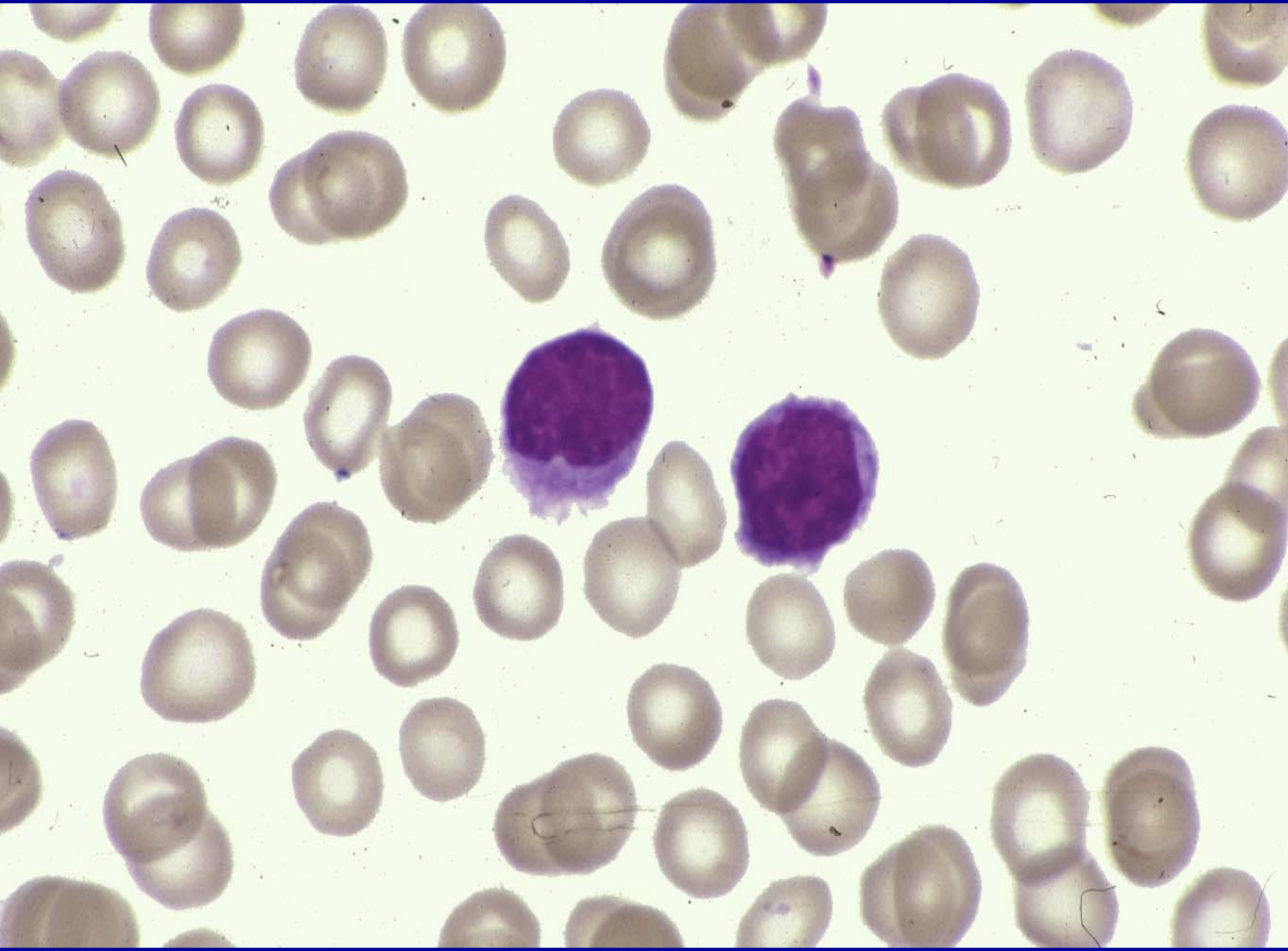
Morphology should describe: cell size, nucleolus, chromatin condensation

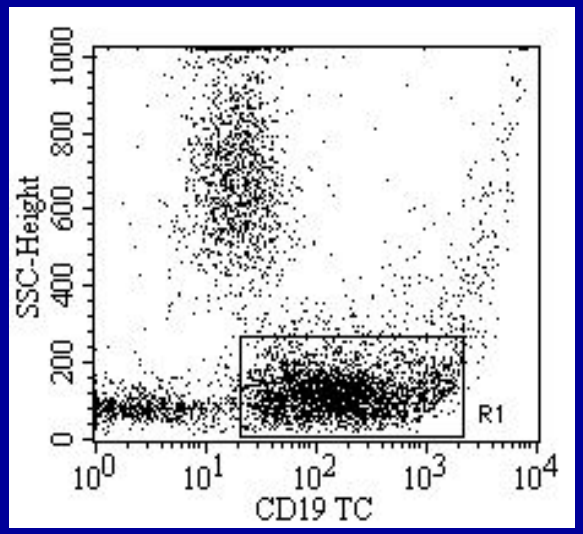
Immunophenotype should include hairy cell associated markers: CD25, CD11c, CD103 and CD123

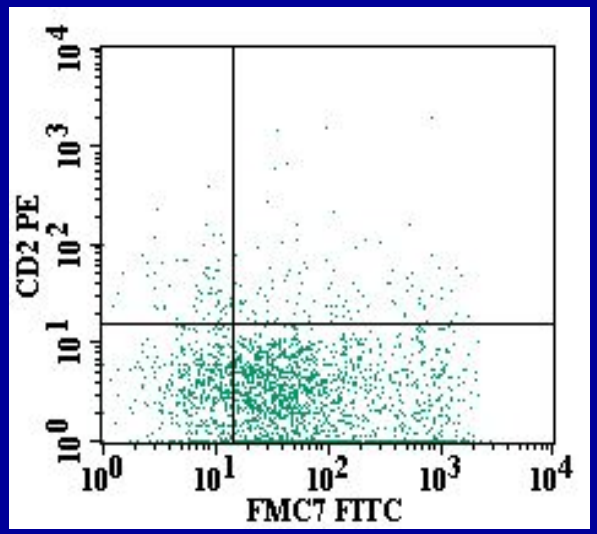
Bone marrow histology: pattern of infiltration (spacing and nodular infiltrates). Intrasinusoidal infiltration or DBA44+ are not specific. HCL (cyclin D1 weakly+ and Annexin A)

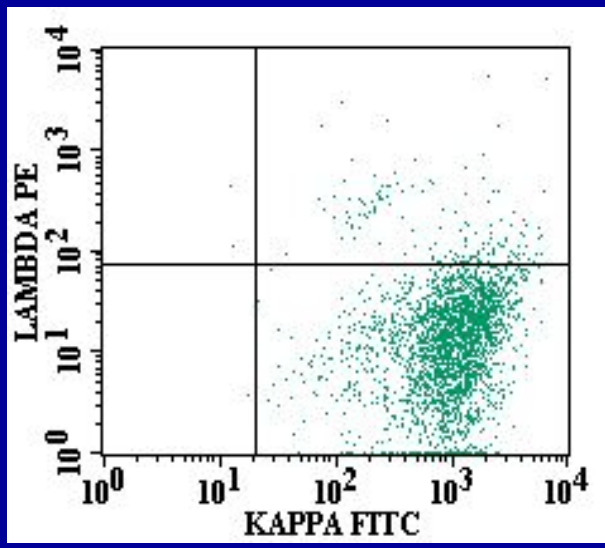
Cytogenetics/FISH: 7q abnormalities, +3/3q, 17p (*TP53*)

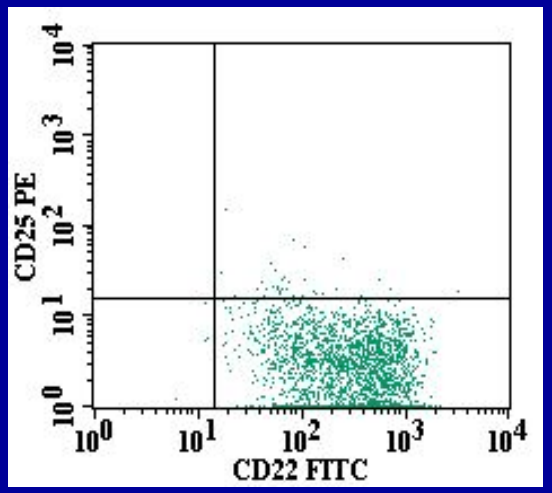
Even sometimes with all this information spleen histology is required to confirm the diagnosis of SMZL and HCL-variant and this should be stated in the report

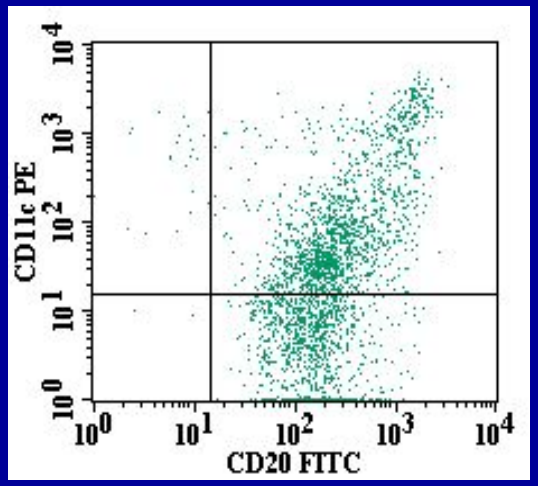


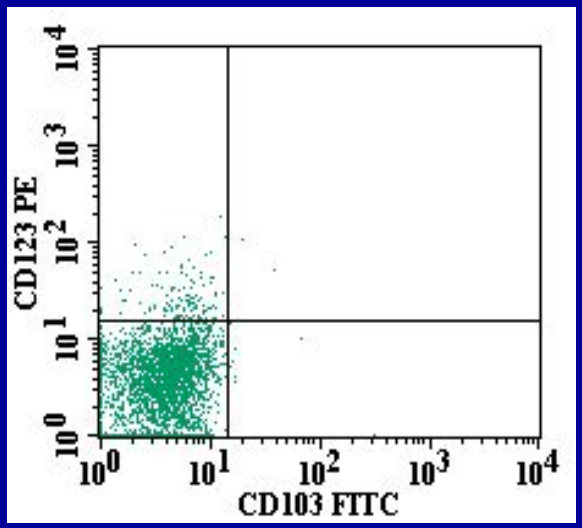




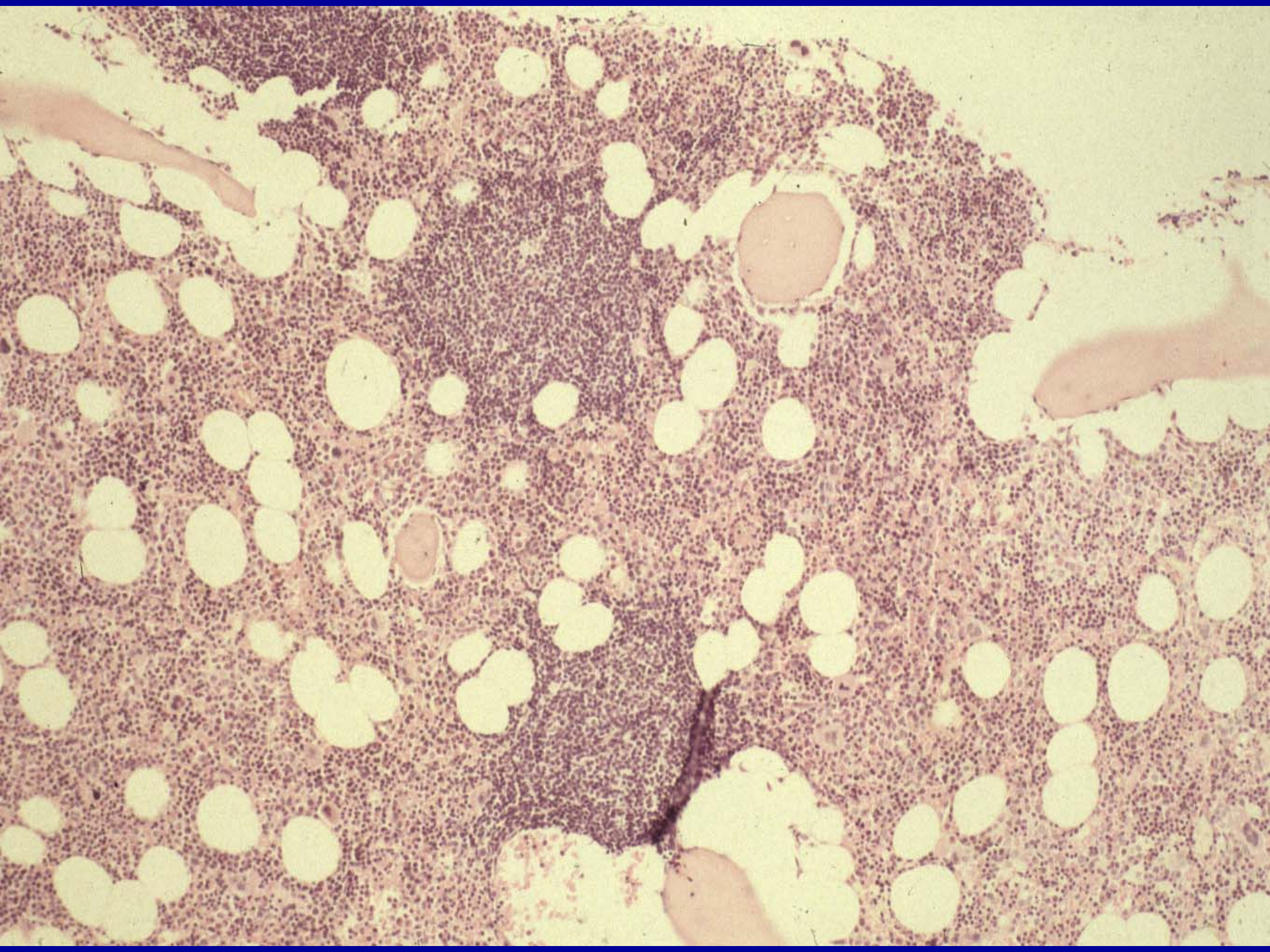


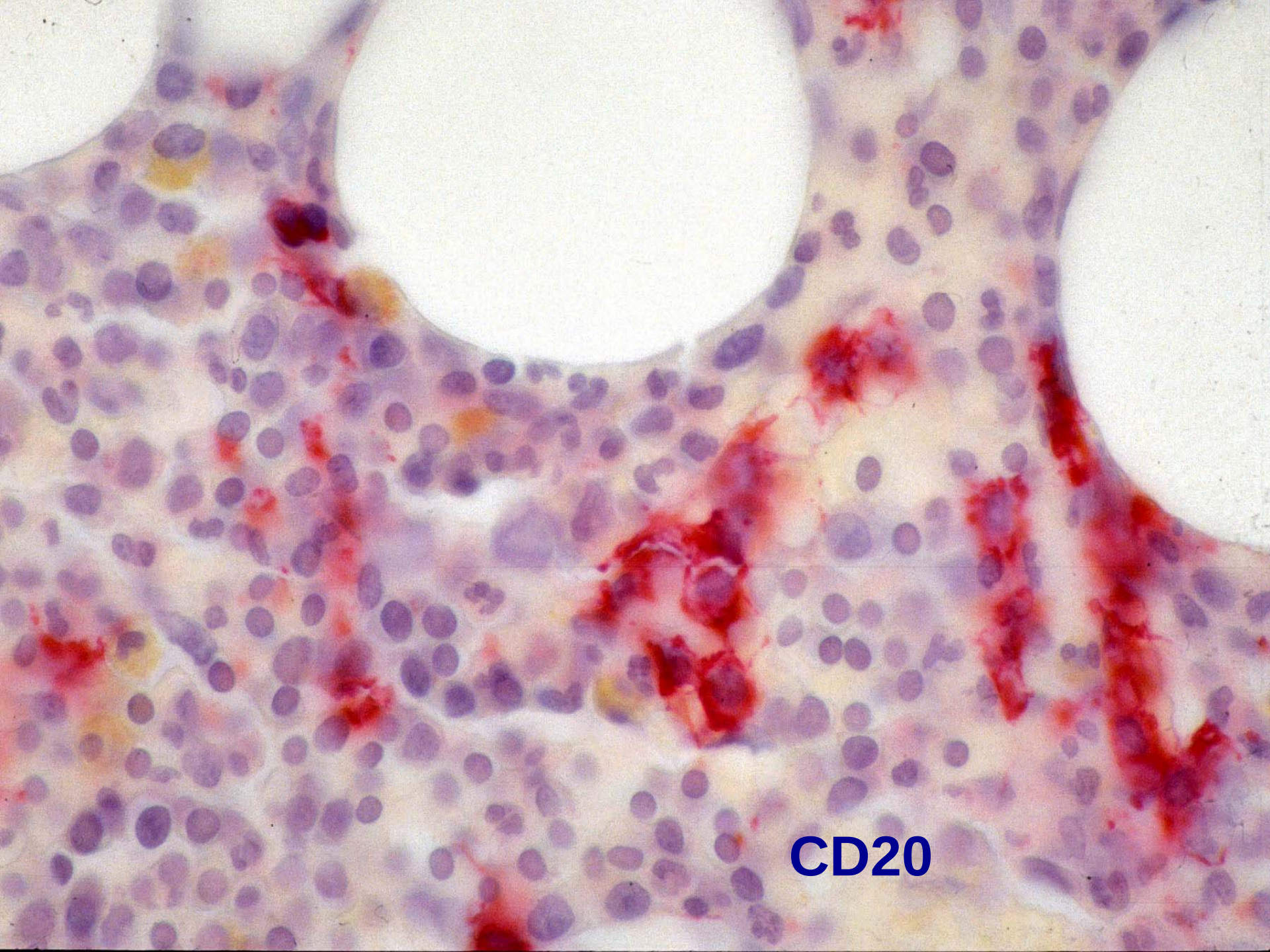






CD19+ Kappa++ CD22+ FMC7+ CD11c+ CD25- CD103- CD123-




CD20

t(2;7)

Integrated report

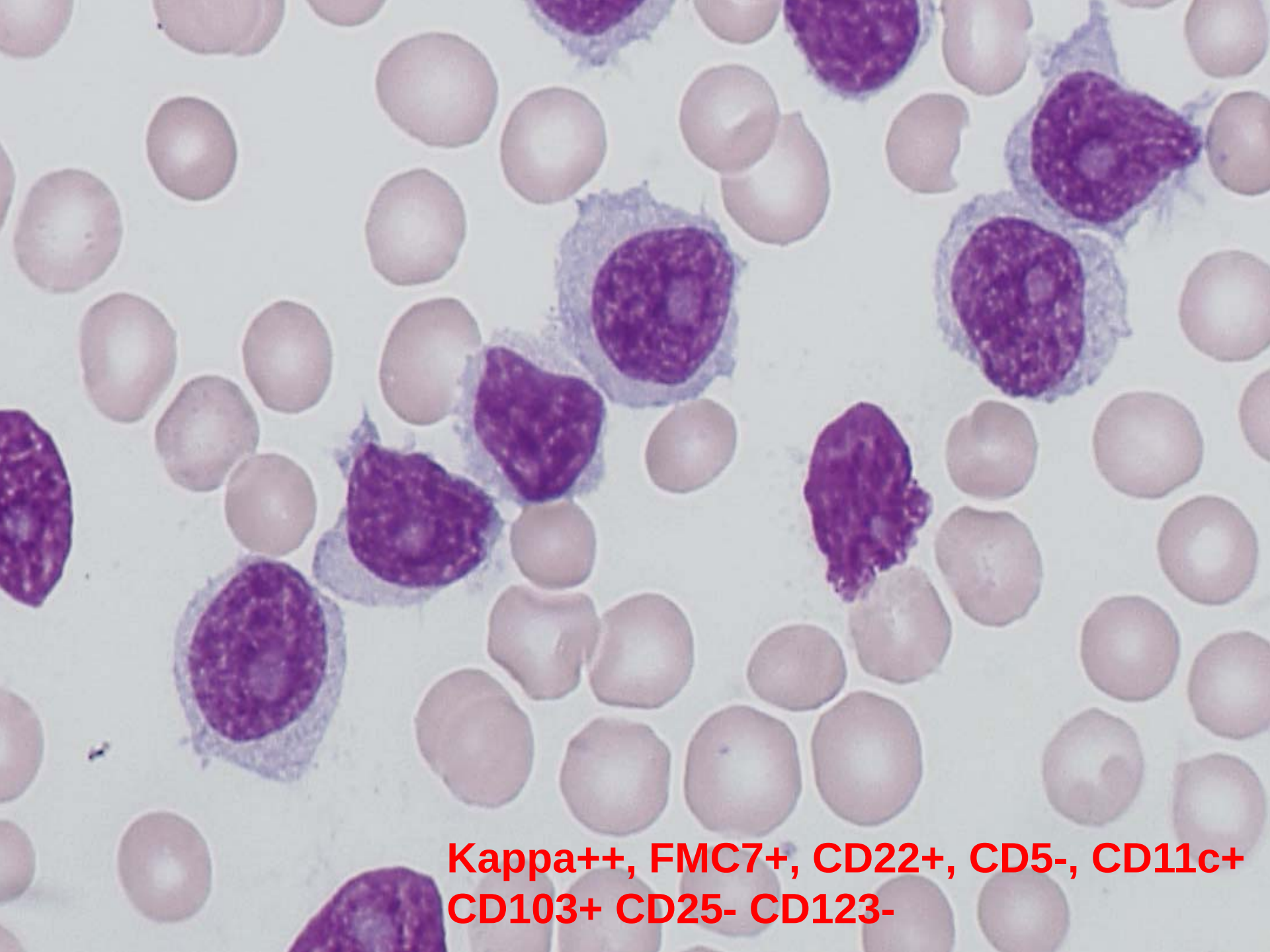
Morphology: small lymphocytes with condensed chromatin, scanty cytoplasm and short thin villi

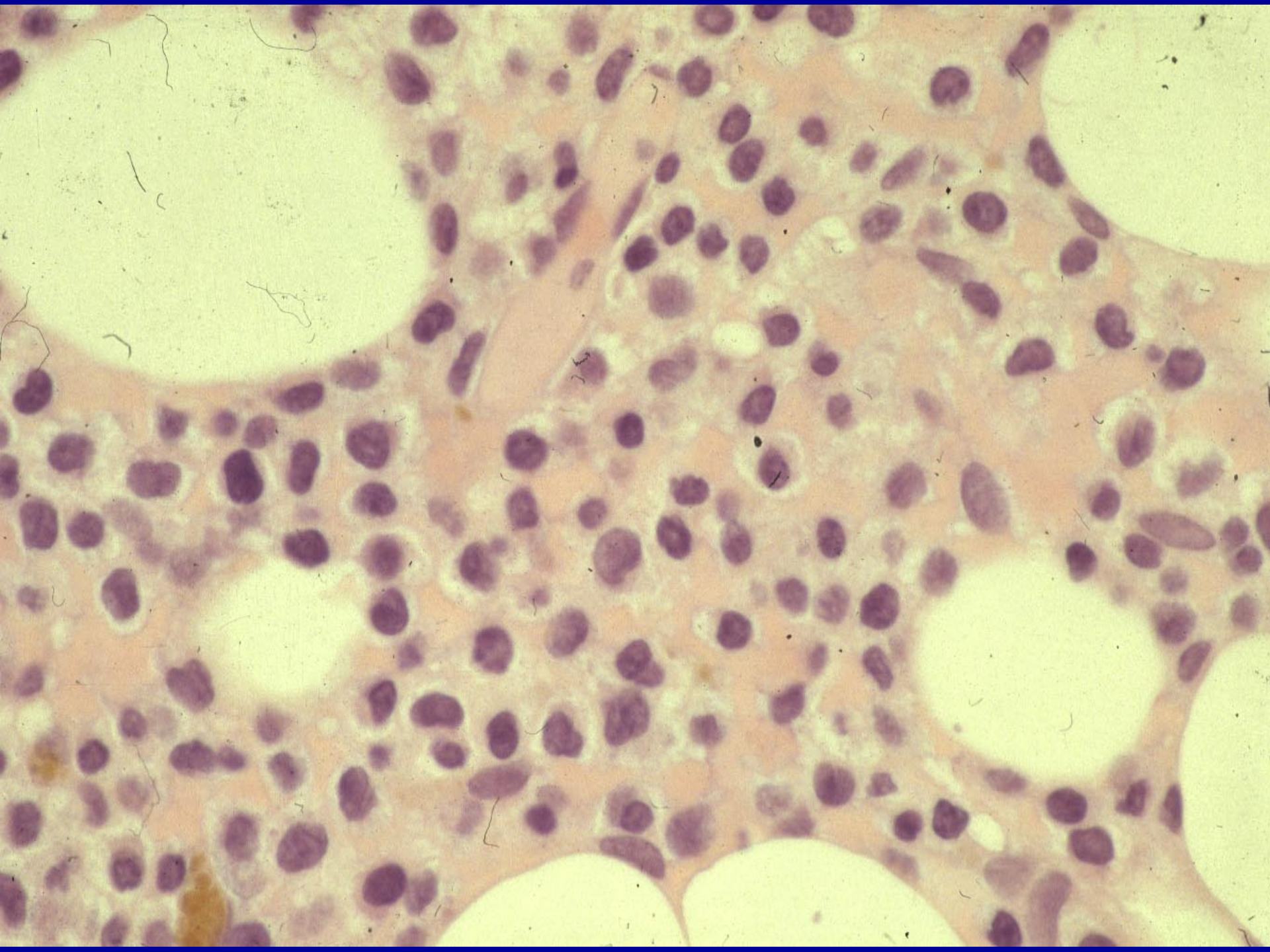
Immunophenotype : Clonal B-cells, CD5-, CD23-, CD25-, CD11c+ and negative with CD103 and CD123

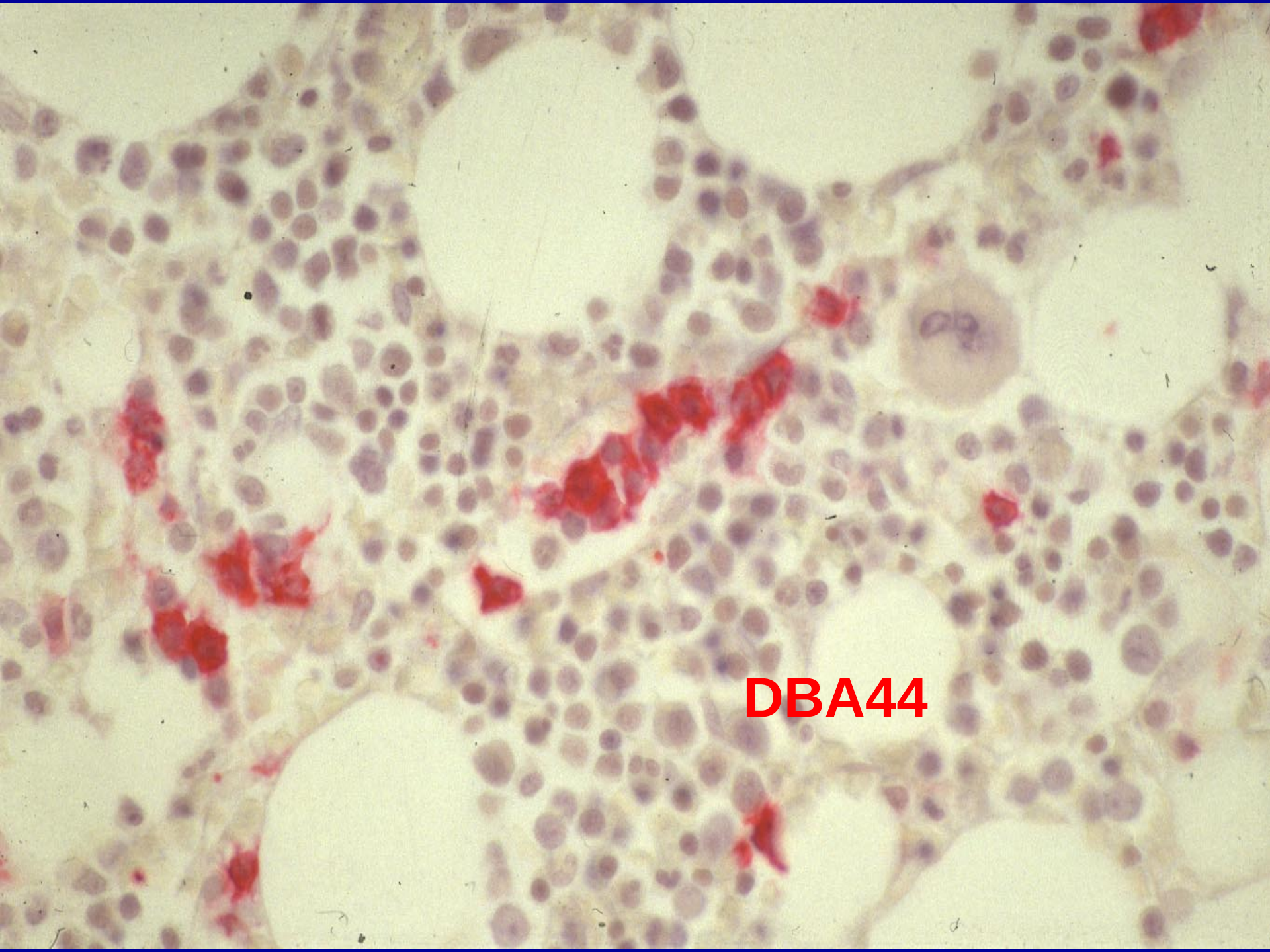
Bone marrow histology: Intrasinusoidal and interstitial infiltration with a few intertrabecular lymphoid aggregates

Cytogenetics/FISH: t(2;7) (p12;q21-22) and 3q+. No evidence of *TP53* deletion or t(11;14) or t(14;18)

Diagnosis: SMZL/SLVL


Kappa++, FMC7+, CD22+, CD5-, CD11c+
CD103+ CD25- CD123-




DBA44

Integrated report

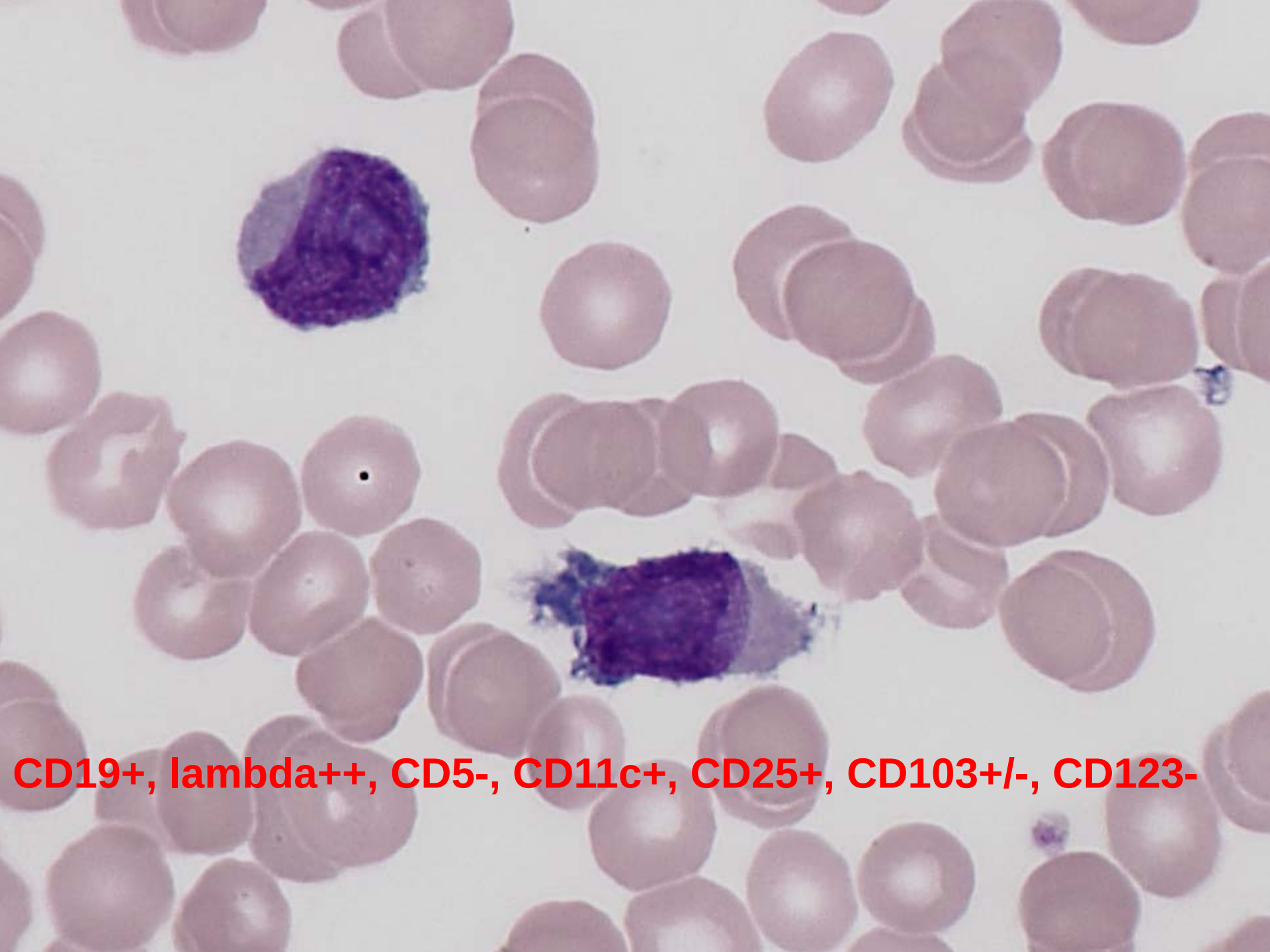
Morphology: medium size lymphocytes with abundant cytoplasm with short villi and prominent vesicular nucleolus

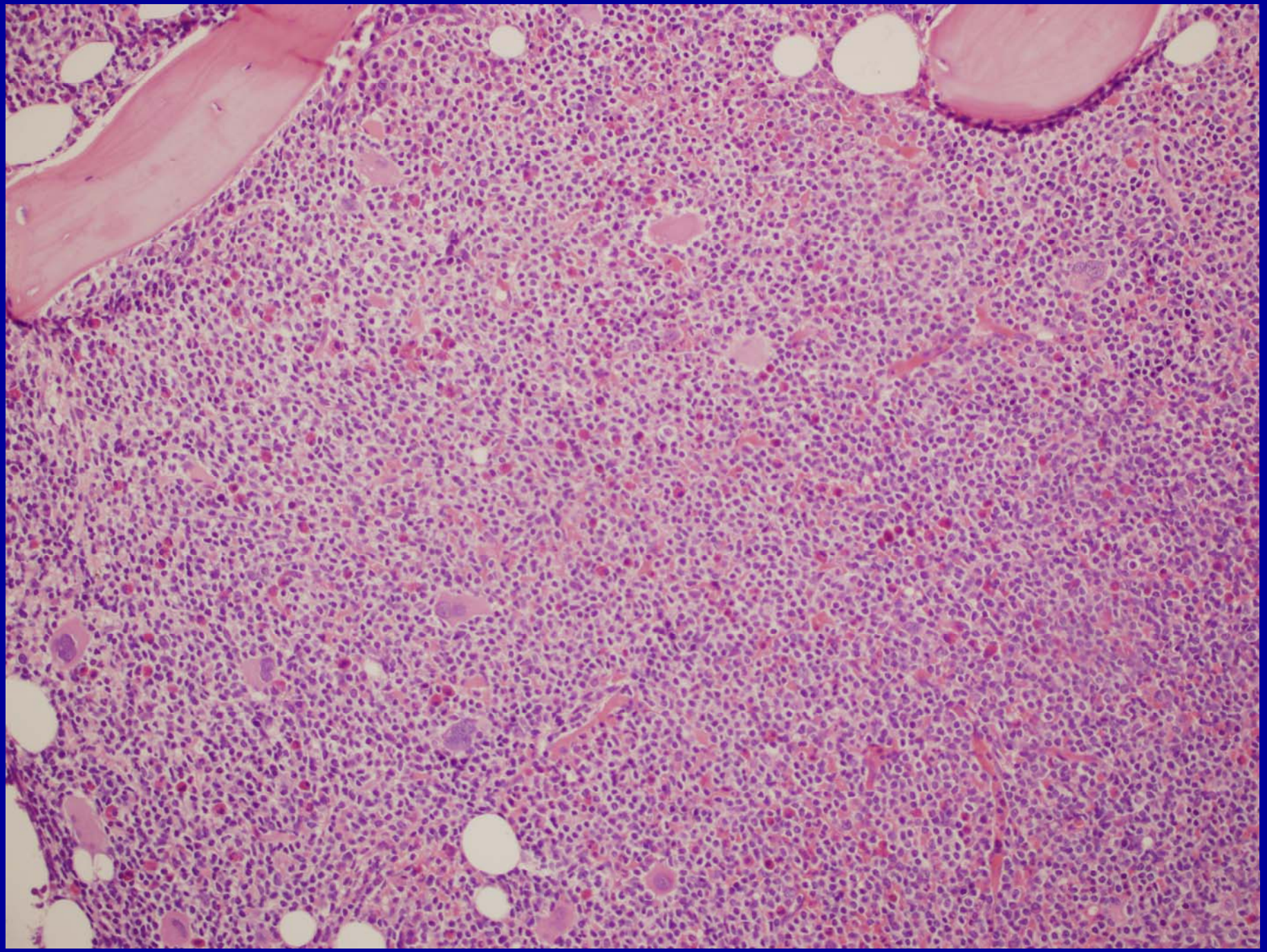
Immunophenotype : Clonal B-cells, CD5-, CD23-, CD25-, CD11c+ CD103+ and negative CD123

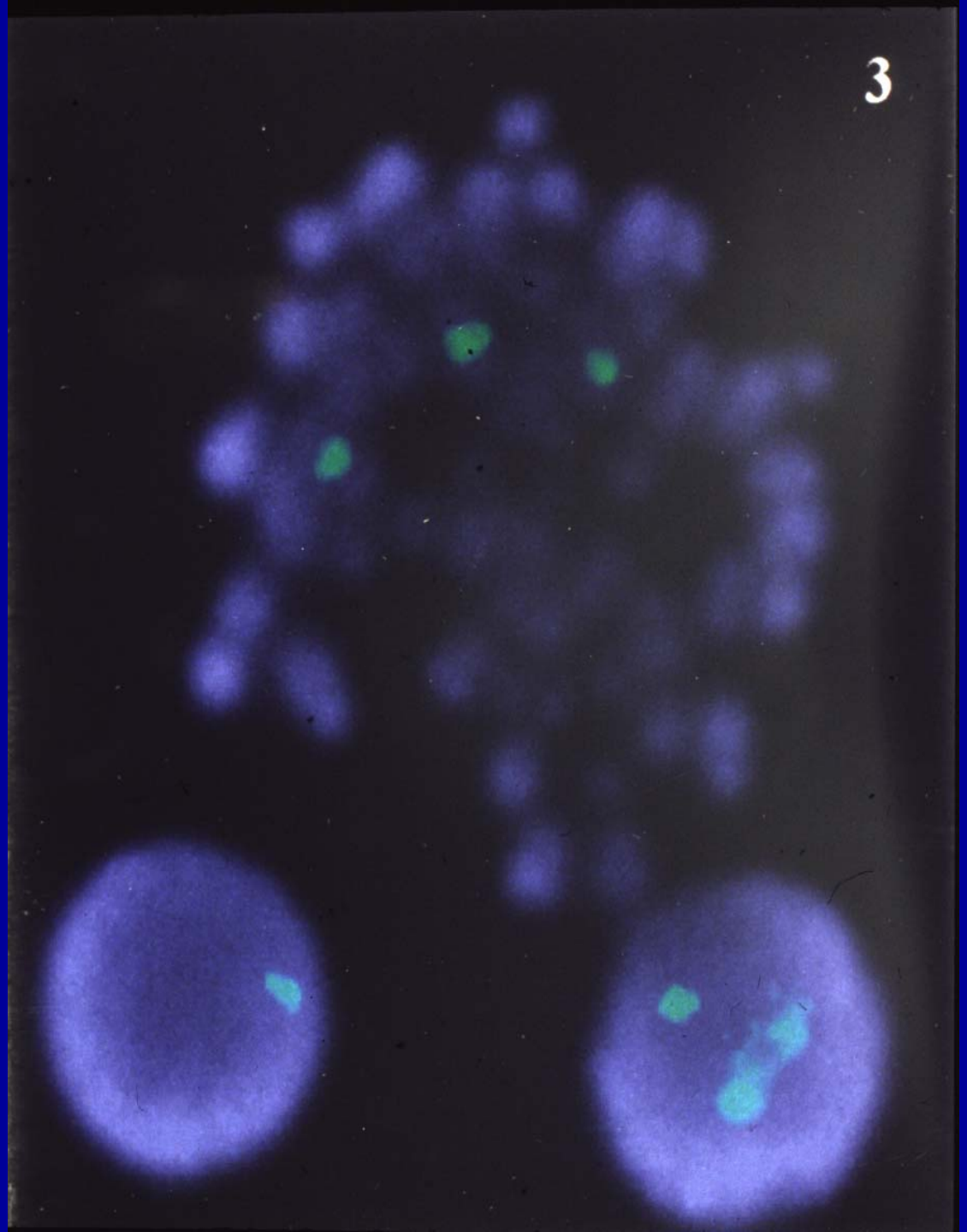
Bone marrow histology: Intrasinusoidal and interstitial infiltration with spacing

Cytogenetics/FISH: del 17p *(TP53)*

Diagnosis: HCL-variant


CD19+, lambda++, CD5-, CD11c+, CD25+, CD103+/-, CD123-




3

Integrated report

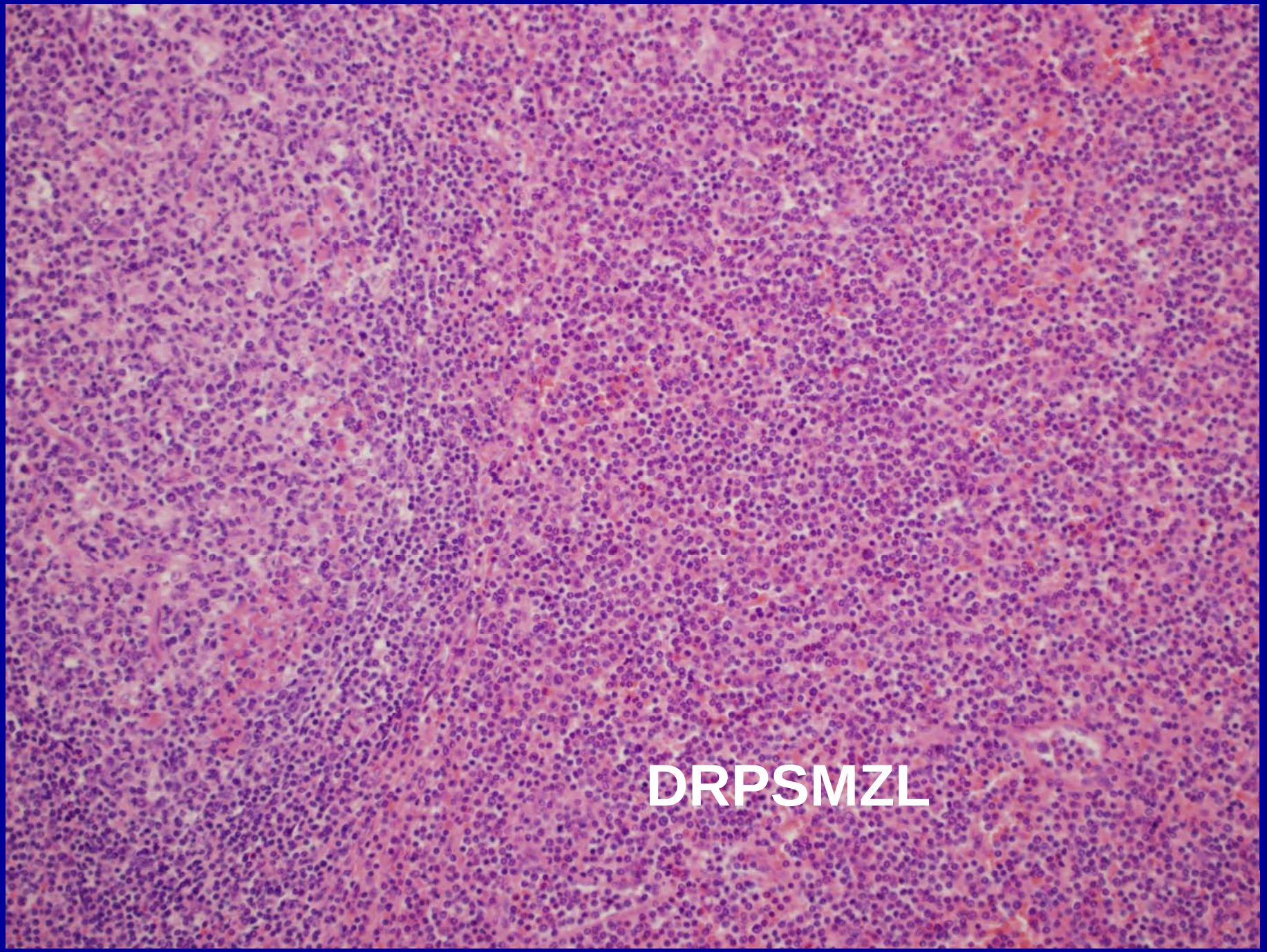
Morphology: small lymphocytes with condensed chromatin, basophilic cytoplasm with short thin villi and a few nucleolated

Immunophenotype : Clonal B-cells, CD5-, CD23-, CD25+, CD11c+ CD103+/- and CD123 negative

Bone marrow histology: Diffuse infiltration by lymphoid cells; no spacing

Cytogenetics/FISH: Trisomy 3. No evidence of TP53 deletion or t(11;14) or t(14;18)

Diagnosis: Likely SMZL but spleen histology will help for a final diagnosis (? Diffuse red pulp SMZL)


DRPSMZL

CLPD of T and NK cells

Immunophenotype

Essential to establish the T/NK-cell origin by flow cytometry in leukaemic forms or IHC in nodal/extranodal forms

Morphology

Important in leukaemic forms

Histology

Essential in nodal/extranodal/cutaneous forms

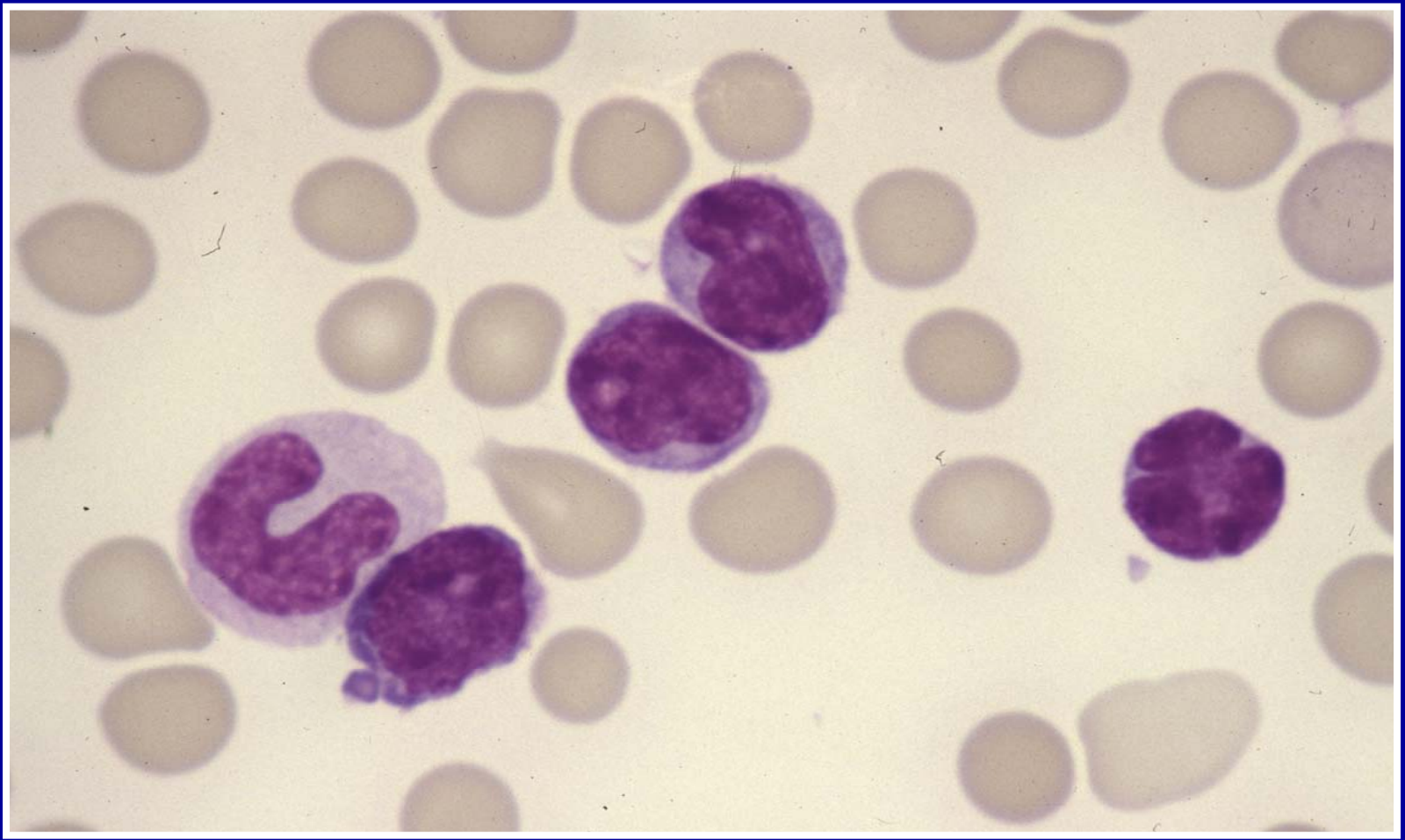
HTLV-I status (serology, PCR, Southern blot)

Suspected ATLL and PTCL unspecified

Molecular genetics

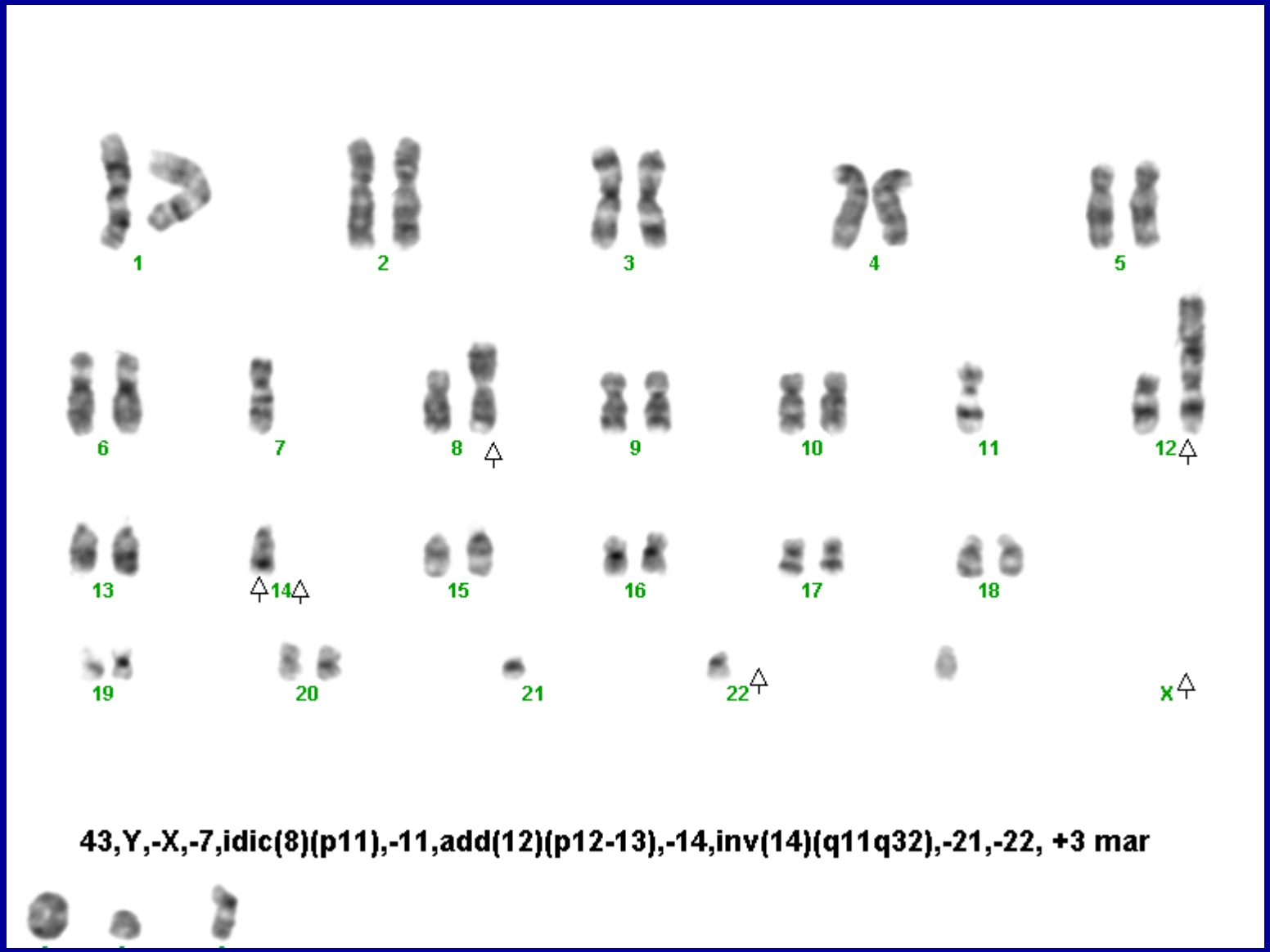
Indolent or chronic “T-cell lymphocytosis”, in difficult or problematic cases and/or rarely for prognosis

Three examples of patients with T-cell lymphocytosis referred to for diagnostic purposes



CD2+ CD3-/+ CD7++ CD4+ CD8+ TdT- CD25+/- CD57-

Complex karyotype



43,Y,-X,-7,idic(8)(p11),-11,add(12)(p12-13),-14,inv(14)(q11q32),-21,-22, +3 mar

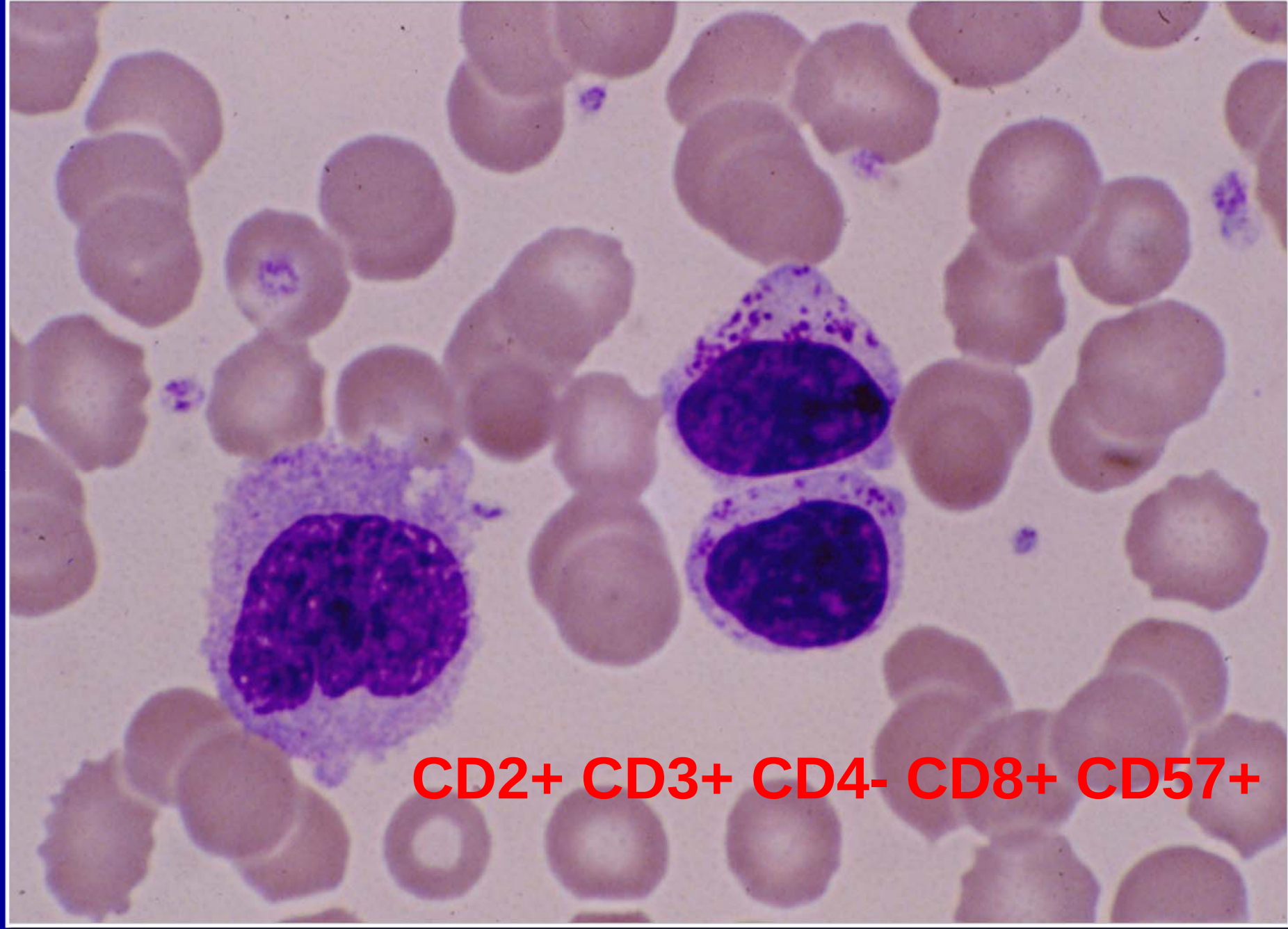
Integrated report

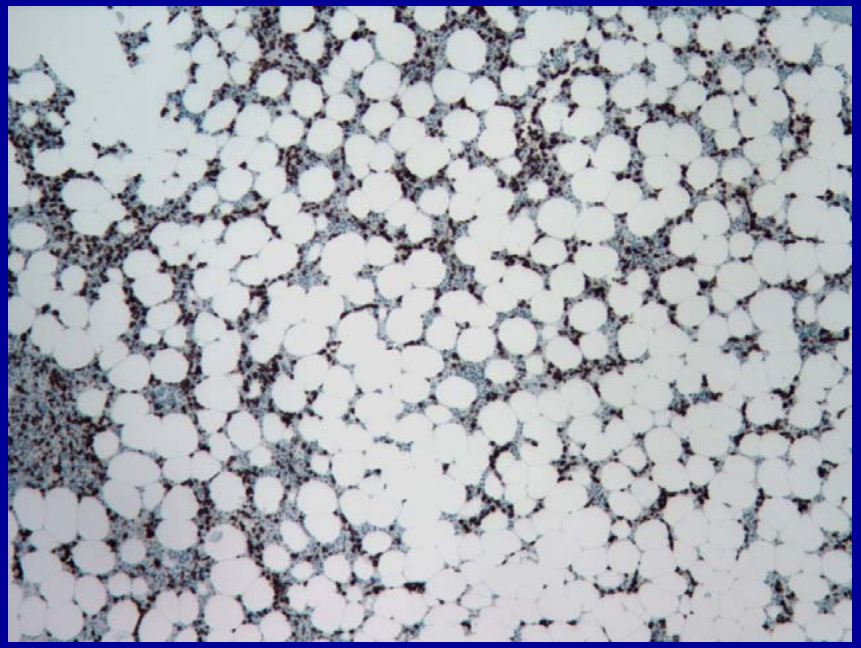
Morphology: small lymphocytes with condensed chromatin, occasionally irregular nucleus with small nucleolus

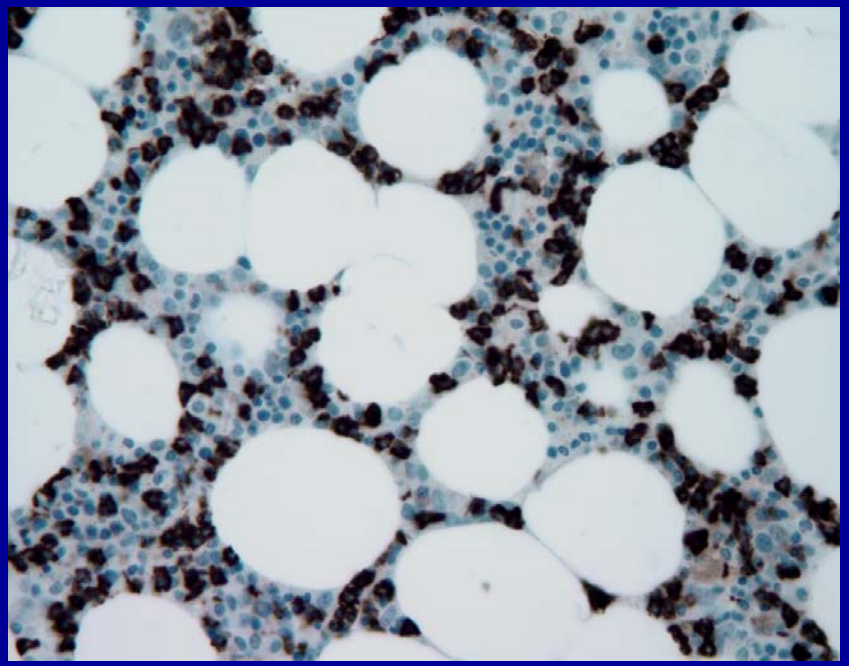
Immunophenotype : Mature T-cells with an atypical phenotype CD2+ CD3- CD25+, strong expression of CD7 and co-expression of CD4&CD8

Cytogenetics: Complex clonal genetics abnormalties including inv(14)(q11;q32) and a idic (8)(p11)

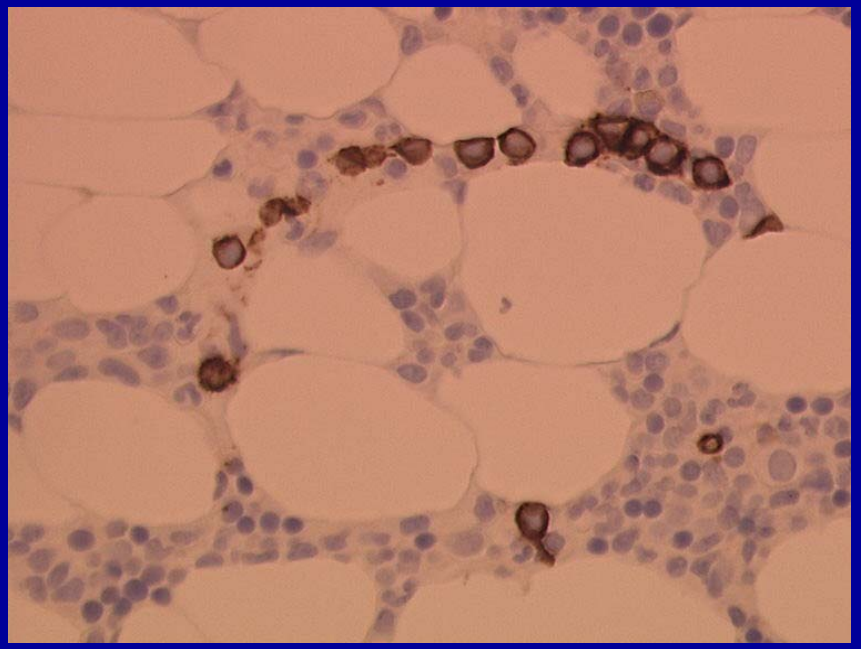
Diagnosis: T-cell prolymphocytic leukaemia (small cell variant)


CD2+ CD3+ CD4- CD8+ CD57+

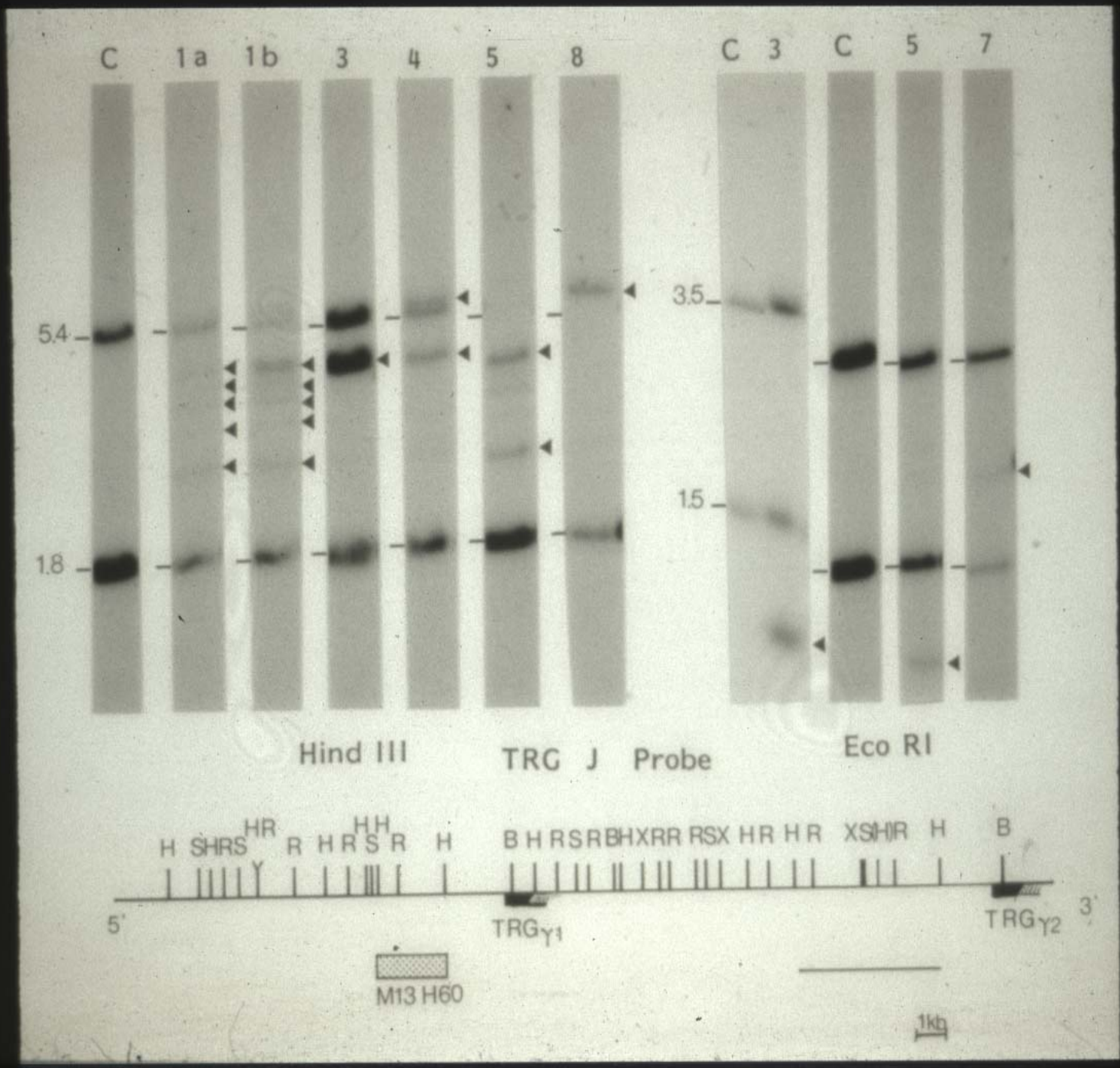




Interstitial&Intrasinusoidal



CD8



Line 3 Patient

Integrated report

Morphology: lymphocytes with condensed chromatin, abundant cytoplasm with azurophilic granulation

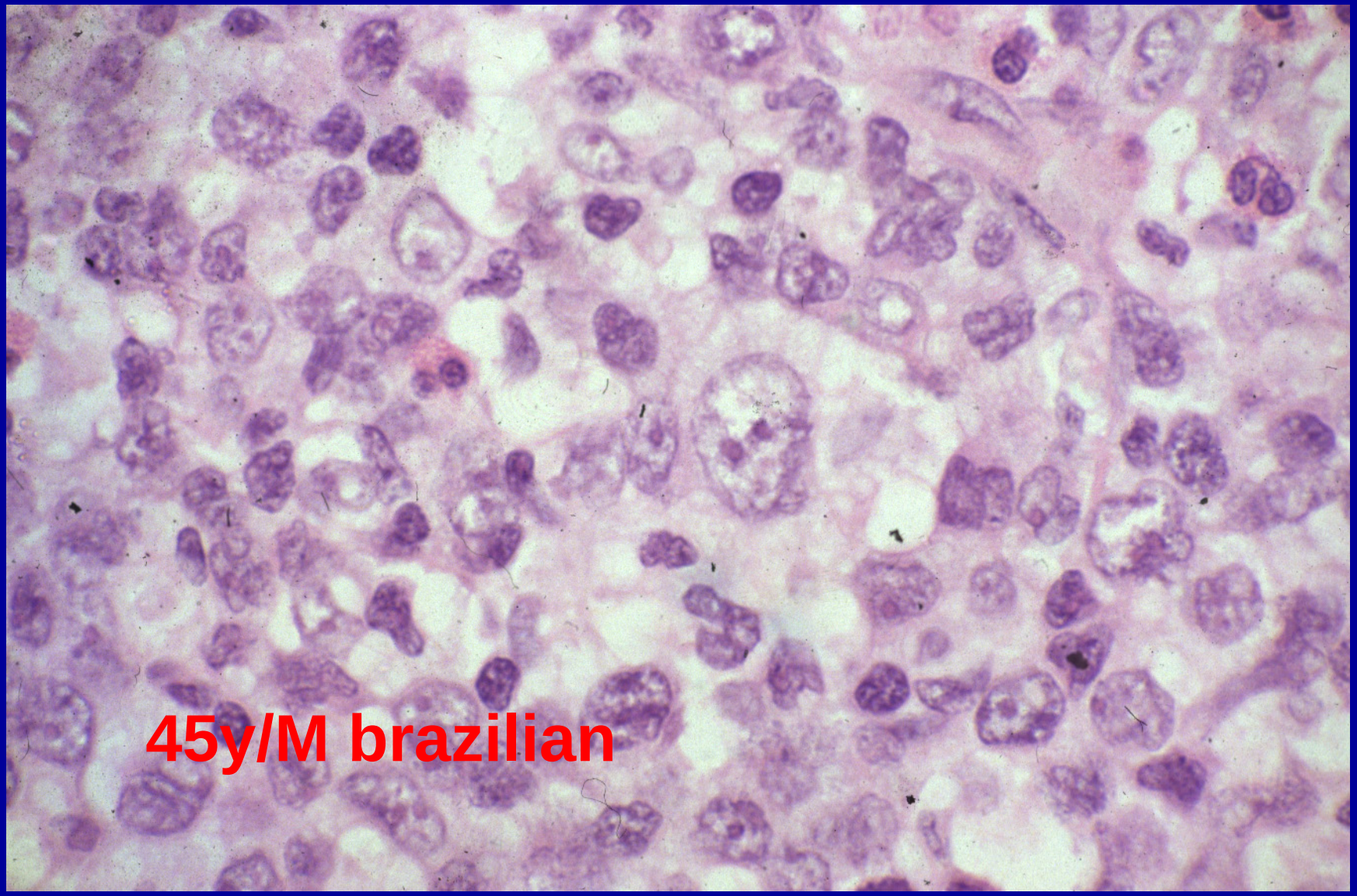
Immunophenotype : Mature T-cells with a phenotype CD2+ CD3+ CD7- CD8+ CD57+ characteristically found in LGL cells

T-cell proliferation ?reactive ?clonal entertained

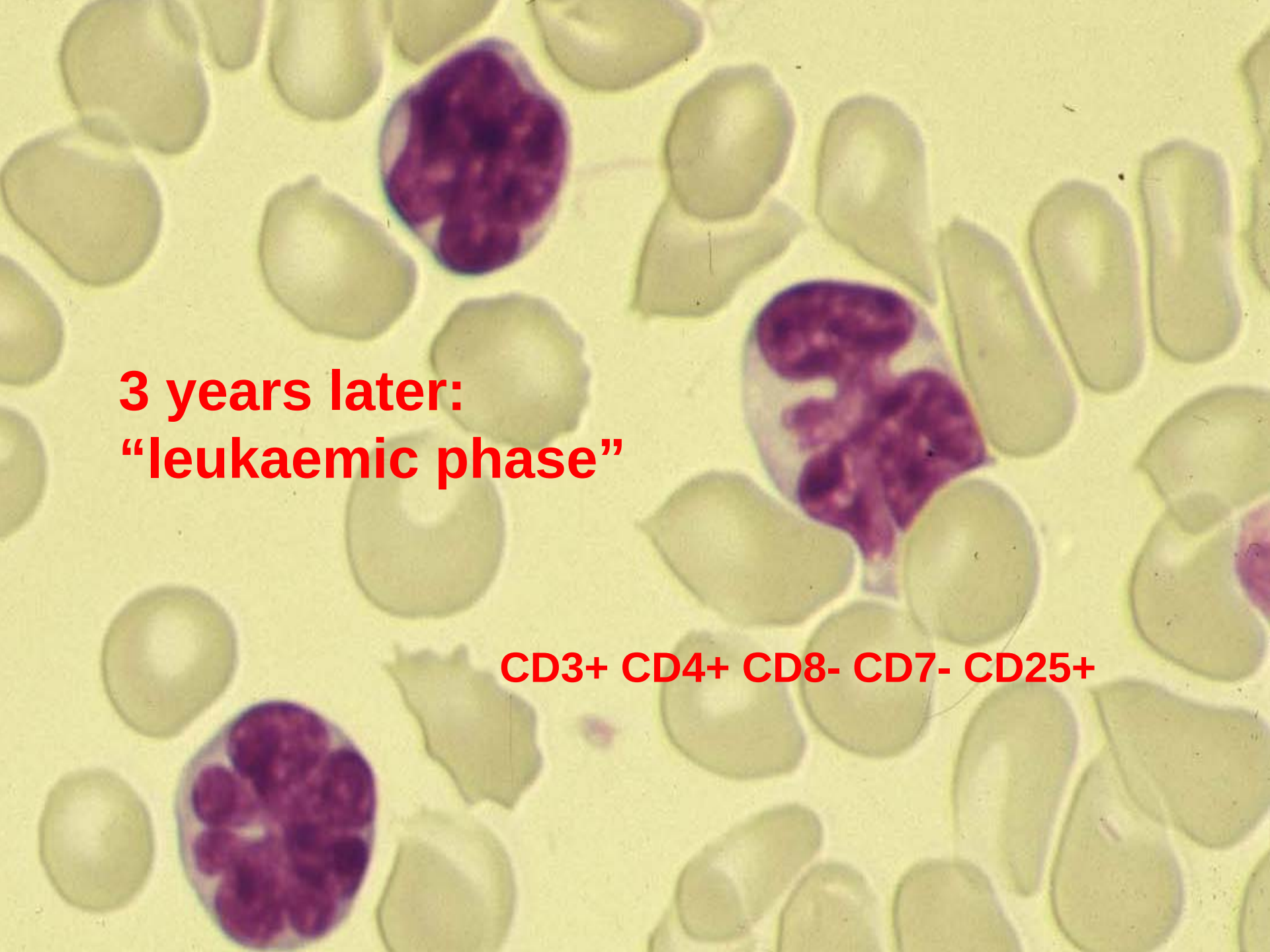
Bone marrow: Interstitial and intrasinusoidal CD8+ infiltrates with reactive nodules and good haemopoietic reserve

DNA analysis for the TCR chain genes: Rearrangement of the TCR gamma chain gene

Diagnosis: T-cell large granular lymphocytic leukaemia



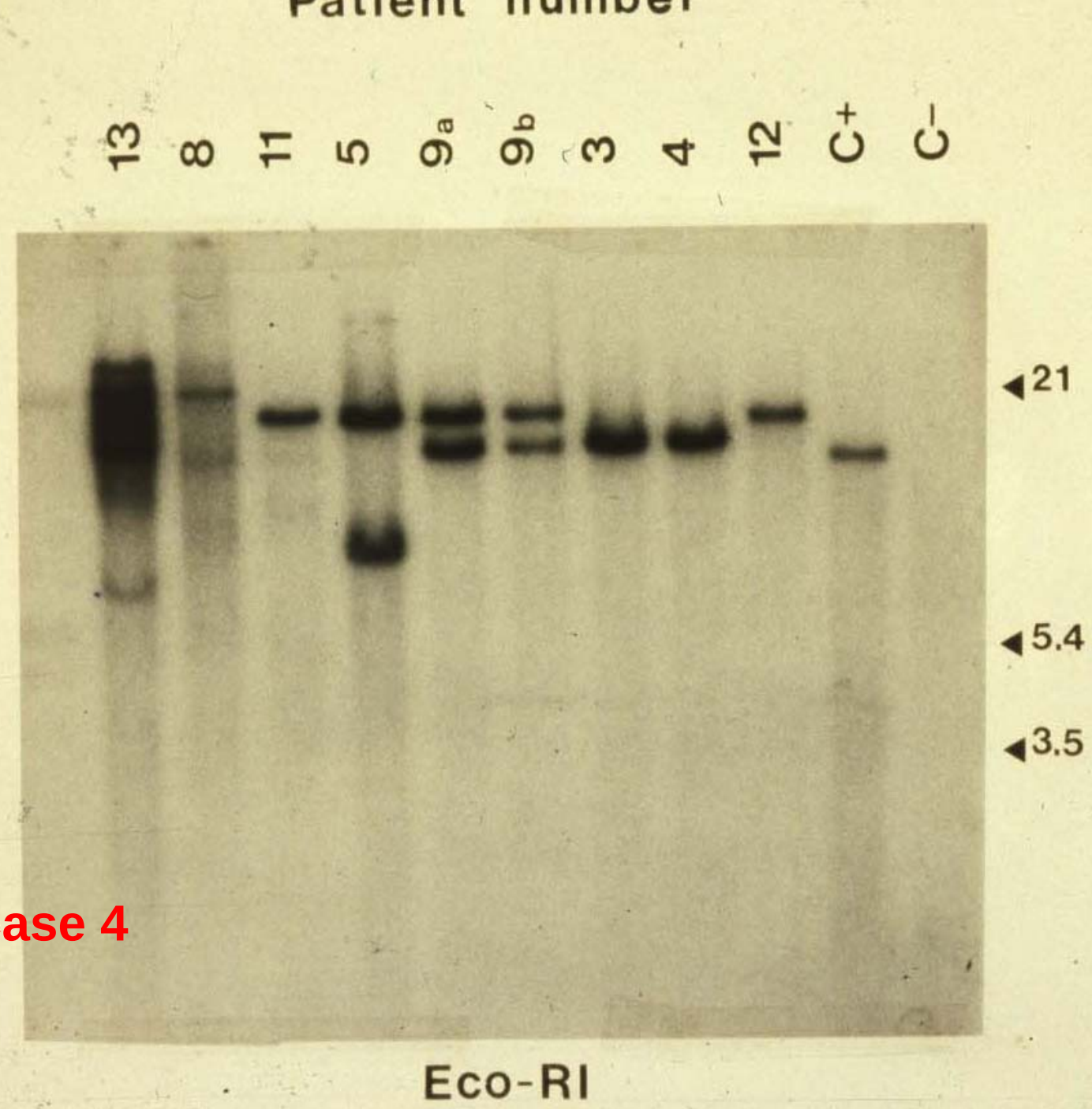
Diagnosis in 2005: PTCL NOS in node
CD3+ CD4+ CD8-


3 years later:
“leukaemic phase”
CD3+ CD4+ CD8- CD7- CD25+

Which test do you think needs to be carried out before an integrated report is made?

Which test do you think needs to be carried out before an integrated report is made?

HTLV-1 serology and/or Southern blot/PCR analysis


Patient number
13
8
11
5
9a
9b
3
4
12
C+
C−
21
5.4
3.5
Case 4
Eco-RI

Integrated report

Morphology: circulating lymphocytes with condensed chromatin and convoluted nucleus (flower cells)

Immunophenotype : Mature T-cells with an atypical phenotype CD2+ CD3- CD7- CD4+ CD25++

Histology review of lymph node: diffuse infiltration by pleomorphic T cells (CD4+ CD8-)

Southern blot analysis in the blood and PCR in lymph node with probes/primers specific for HTVL-1: Clonal integration of HTVL-1 in the lymphocyte´s DNA

Diagnosis: ATLL (HTLV-1+) originally manifesting with a lymphoma subtype that evolved into a leukaemic form

Conclusions

The integrated report in CLPD should provide the clinician diagnostic and when applicable prognostic information

It should “integrate” the results from comprehensive investigations

A mutidisciplinary team should be involved in the interpretations of the data to issue the report

There may be difficult cases even when all the investigations have been performed. In such situation is advisable to describe findings and possible diagnosis without being categoric